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16. ABSTRACT

A single intramuscular injection of nickel chloride (18.3 mg/kg) caused a significant reduction in murine splenic natural killer (NK) cell activity. This reduction in NK activity was not associated with a significant reduction in spleen cellularity nor in the production of suppressor cells. In a 4-hr ^{51}Cr release assay, NK cell activity was suppressed in both CBA/J and C57BL/6J mice. Administration of the nickel dose (i.e., 18.3 mg/kg total) over a two week period also caused a significant reduction in NK cell activity. In an *in vivo* NK assay the clearance of ^{125}I-iododeoxyuridine labeled YAC-1 tumor cells from the lungs of nickel-treated mice was significantly reduced compared with saline injected controls. Another *in vivo* correlate of nickel-induced NK suppression was observed in mice injected with the B16-F10 melanoma. Mice given a single intramuscular injection of $NiCl_2$ (18.3 mg/kg) developed significantly greater numbers of lung tumors than saline controls. The results indicate that $NiCl_2$ is a potent suppressor of NK cell activity.

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Immunologic Effects of Nickel

II. Suppression of Natural Killer Cell Activity

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A single intramuscular injection of nickel chloride (18.3 mg/kg) caused a significant reduction in murine splenic natural killer (NK) cell activity. This reduction in NK activity was not associated with a significant reduction in spleen cellularity nor in the production of suppressor cells. In a 4-hr ^{51}Cr-release assay, NK cell activity was suppressed in both CBA/J and C57BL/6J mice. Administration of the nickel dose (i.e., 18.3 mg/kg total) over a 2-week period also caused a significant reduction in NK cell activity. In an *in vivo* NK assay, the clearance of [^{125}I]iododeoxyuridine-labeled YAC-1 tumor cells from the lungs of nickel-treated mice was significantly reduced compared with saline injected controls. Another *in vivo* correlate of nickel-induced NK suppression was observed in mice injected with the B16-F10 melanoma. Mice given a single intramuscular injection of $NiCl_2$ (18.3 mg/kg) developed significantly greater numbers of lung tumors than saline controls. The results indicate that $NiCl_2$ is a potent suppressor of NK cell activity. © 1985 Academic Press, Inc.

INTRODUCTION

There is increasing evidence that nickel compounds can adversely affect the immune system of experimental animals (for reviews see Vos, 1977; Koller, 1979, 1980). The immunosuppressive effects of nickel compounds include depression of interferon production *in vitro* (Treagan and Furst, 1970) and *in vivo* (Gainer, 1977). Nickel compounds have also been reported to enhance the infectivity of encephalomyocarditis virus (Gainer, 1977) and *Streptococcus pyogenes* (Adkins *et al.*, 1979) in rodents. Inhibition of macrophage phagocytic function (Graham *et al.*, 1975) and suppression of antibody responses in rodents treated with nickel compounds have also been reported (Figoni and Treagan, 1975; Graham *et al.*, 1978; Smialowicz *et al.*, 1984). We also reported that a single parenteral injection of nickel chloride caused a suppression of T-lymphocyte-mediated reactions and reduced natural killer cell activity in treated mice (Smialowicz *et al.*, 1984).

Natural killer (NK) cells have been receiving increased attention in recent years because of their potential role as primary cytotoxic effector cells against tumors. NK cells display spontaneous *in vitro* and *in vivo* cytotoxicity against tumor cells without immunologic priming (Herberman and Ortaldo, 1981; Kiessling and Wigzell, 1979). It has been suggested that they are one of the first lines of defense

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against newly arising tumors and they appear to play a role as *in vivo* effectors against transplanted tumors (Kasai *et al.*, 1979; Hanna and Burton, 1981). There is also increasing evidence that NK cells play a role in resistance to certain viral and protozoan infections (Herberman and Ortaldo, 1981).

In the present paper we have focused on the effect of $NiCl_2$ on NK cell activity in mice. We show that suppression of NK activity by nickel can be detected by both *in vitro* and *in vivo* assays; that suppression of NK activity by nickel is not mediated by suppressor cells; and that $NiCl_2$ suppressed NK activity may lead to an increase in the development of transplanted tumors.

MATERIALS AND METHODS

Mice

Eight-week-old male CBA/J and female C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, Maine). Mice were maintained in our animal facilities for at least 1 week after arrival to allow recovery from shipping. All mice were used by 14 weeks of age.

Nickel Chloride

Nickel chloride was prepared as a sterile stock solution in saline as previously described (Smialowicz *et al.*, 1984). The stock solution was found to contain 18.3 mg $NiCl_2$/ml as analyzed by neutron activation analysis. Dilutions for injection were prepared in sterile saline and injected intramuscularly (im) in the thigh on a body weight basis in a volume of less than 0.15 ml. Control mice were injected with sterile saline.

In Vitro NK Assay

Spleen cell suspensions (effector cells) were prepared in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 25 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (Hepes), 100 units/ml penicillin, and 100 μg/ml streptomycin (Grand Island Biological Co., Grand Island, N.Y.). Erythrocytes were lysed by adding 0.83% NH_4Cl. Cell suspensions were washed and resuspended to 2×10^7 viable cells/ml. Viability was determined by trypan blue exclusion.

Target cells, YAC-1 murine lymphoma cells, were labeled with ^{51}Cr (50 μCi ^{51}Cr as sodium chromate/10^6 cells, sp act 200 to 500 Ci/g, New England Nuclear Corp., Boston, Mass.) for 1 hr at 37°C. The cells were washed three times in RPMI/FBS and resuspended to 2×10^5 cells/ml. Labeled target cells (2×10^4 cells/100 μl) were cultured with graded numbers of splenic effector cells (100 μl) in round-bottom microtiter plates (Linbro, Flow Labs, Rockville, Md.). Three replicates of each effector-to-target-cell ratio (25:1, 50:1, and 100:1) were incubated for 4 hr at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. After incubation, the plates were centrifuged for 5 min at 250*g* and the supernatants were collected using the Titertek supernatant collection system (Flow Labs). Percentage specific ^{51}Cr release was determined from the formula: $[(E-S)/(T-S)] \times 100$ where E is the ^{51}Cr released from target cells in the presence of spleen cells, S is the spontaneous release of ^{51}Cr from target cells alone, and T is the maximum release of ^{51}Cr from target cells in the presence of 0.25% Triton X-100.

Spontaneous release was typically less than 10% of the maximum releasable ^{51}Cr activity.

In Vivo NK Assay

In vivo NK activity was evaluated according to the procedure described by Riccardi *et al.* (1980). YAC-1 tumor cells were labeled with [^{125}I]5-iodo-2′ deoxyuridine (New England Nuclear) in the presence of 5-fluoro-2′-deoxyuridine, washed, and resuspended to 5×10^6 cells/ml in unsupplemented RPMI medium. One million labeled cells were then injected into the tail vein of each mouse. Mice were killed 4 hr later by cervical dislocation and their spleens and lungs were removed, placed in tubes and organ radioactivity was measured. Results are expressed as percentage recovery (geometric mean) of injected radioactivity.

Assay for Suppressor Cell Activity

Assay for putative suppressor cells was performed as described by Hochman and Cudkowicz (1979). Graded numbers of spleen cells ($0.25–2.0 \times 10^6$) from $NiCl_2$-treated mice were added to an equal volume of splenic effector cells (1×10^6) from saline-injected mice. Volumes of 25, 50, or 100 μl of these cell suspensions were added to round-bottom wells of microtiter plates and the volume of each well was then brought up to 100 μl with RPMI/FBS medium. To each well was added 100 μl of ^{51}Cr-labeled YAC-1 targets (2×10^4) giving final effector-to-target-cell (E:T) ratios of 25:1, 50:1, and 100:1. The plates were then handled exactly as described for the NK cytotoxicity assay. In place of splenocytes from $NiCl_2$-treated mice, thymus cells from saline-injected mice were used as filler cells in mixture with effectors and targets to monitor the nonspecific effect of adding "third party" cells in the assay. Therefore, in order to attribute inhibition of NK lysis to suppressor cells from $NiCl_2$-treated mice the magnitude of inhibition would have to significantly exceed that which was caused by the filler cells (Hochman and Cudkowicz, 1979).

B16-F10 Tumors

The development of B16-F10 lung tumors in mice following $NiCl_2$ treatment was determined as described by Hanna and Burton (1981). B16-F10 melanoma tumor cells (obtained from Dr. I. Fidler, National Cancer Institute, Frederick, Md.) were grown in monolayer cultures maintained in Eagle's minimum essential medium (MEM) supplemented with 10% FBS, sodium pyruvate, nonessential amino acids, L-glutamine, and two-fold vitamin solution (CMEM, GIBCO). Exponentially growing cells were harvested from cultures by overlaying monolayers with a solution of 0.25% trypsin–0.02% EDTA for 1 min. The cells were washed in CMEM and resuspended in MEM to 5×10^5 cells/ml. Unanesthetized C57BL/6J mice were injected intravenously with 0.2 ml of B16-F10 cells. The mice were killed 2–3 weeks later, and their lungs were removed and fixed in Bouin's solution. Pulmonary tumor colonies were counted with the aid of a magnifying glass.

Statistical Analysis

Data from the *in vitro* and *in vivo* cytotoxicity assays, and spleen cellularity and cell viabilities were analyzed using either Student's *t* test or Dunnett's *t* test

(two-tailed). The nonparametric Mann–Whitney *U* test (two-tailed) was used to analyze the B16-F10 lung tumor data.

RESULTS

A single intramuscular injection of $NiCl_2$ significantly reduced the NK cell activity of spleen cells from CBA/J and C57BL/6J mice tested 24 hr later (Table 1). Significant ($P < 0.05$) suppression of the NK activity of CBA/J mice (Experiment 1) at all E:T ratios was observed at doses of 18.3 and 27.5 mg/kg. Suppression of NK activity in C57BL/6J mice was observed at doses of 18.3, 27.5, and 36.6 mg/kg (Experiments 2 and 3). Reduced NK activity for spleen cell suspensions from CBA/J mice dosed at 18.3 mg/kg was not associated with a significant decrease in spleen cellularity compared with saline control (Table 2). However, at a dose of 27.5 mg/kg spleen cellularity was significantly reduced ($P < 0.05$, Table 2). This decrease in spleen cellularity was associated with a decrease in NK activity (Table 1). There was no difference in the spleen cell viability among the four CBA/J groups of Experiment 1 with the cell viability for all groups being approximately 94% (Table 2). A reduction was observed in the cellularity of spleens from C57BL/6J mice at all of the $NiCl_2$ doses studied, however, only one of these reductions was significant (Table 2). A significant reduction ($P < 0.05$) in the spleen cellularity of mice injected with 13.7 mg/kg was observed in Experiment 2. This reduction in cellularity however, was not associated with a decrease in NK activity (Table 1, Experiment 2). As observed for the CBA/J mice the spleen cell viability of C57BL/6J mice among the four groups from Experiments 2 and 3 were comparable (Table 2).

In order to determine if the reduced activity of NK cells from $NiCl_2$-treated

TABLE 1
THE EFFECT OF $NiCl_2$ ON NATURAL KILLER CELL ACTIVITY IN CBA/J AND C57BL/6J MICE

Strain	Expt	Treatment[a]	Dose (mg/kg)	$\bar{X}$% ^{51}Cr Release ± SE		
				25:1[b]	50:1	100:1
CBA/J	1	Saline		33.1 ± 2.4	49.4 ± 5.7	59.5 ± 5.3
		$NiCl_2$	9.1	19.5 ± 1.8[d]	30.9 ± 2.5[d]	42.9 ± 9.3
		$NiCl_2$	18.3	15.3 ± 4.5[d]	24.6 ± 5.3[d]	31.4 ± 4.7[c]
		$NiCl_2$	27.5	8.7 ± 2.6[d]	13.3 ± 3.7[d]	17.6 ± 4.5[d]
C57BL/6J	2	Saline		9.1 ± 0.9	14.9 ± 1.6	19.9 ± 1.9
		$NiCl_2$	9.1	10.7 ± 1.1	16.4 ± 1.5	20.5 ± 1.7
		$NiCl_2$	13.7	9.1 ± 0.9	14.7 ± 1.1	18.9 ± 1.7
		$NiCl_2$	18.3	5.1 ± 0.8[d]	7.1 ± 1.6[d]	9.8 ± 2.0[d]
C57BL/6J	3	Saline		8.7 ± 0.7	14.4 ± 0.6	18.4 ± 1.0
		$NiCl_2$	18.3	2.8 ± 0.3[d]	5.1 ± 0.5[d]	6.5 ± 0.7[d]
		$NiCl_2$	27.5	2.7 ± 0.3[d]	4.7 ± 0.4[d]	5.9 ± 0.6[d]
		$NiCl_2$	36.6	1.9 ± 0.3[d]	3.0 ± 0.3[d]	4.0 ± 0.3[d]

[a] Groups of 6–8 mice given a single intramuscular injection 24 hr before assay.
[b] Effector-to-target cell ratio, YAC-1 target.
[c] $P < 0.05$, Dunnett's *t* test compared with saline-injected mice.
[d] $P < 0.01$, Dunnett's *t* test.

TABLE 2
SPLEEN CELLULARITY AND SPLEEN CELL VIABILITY OF CBA/J AND C57BL/6J MICE INJECTED WITH $NiCl_2$

Strain	Expt.	Treatment[a]	Dose (mg/kg)	$\bar{X}$ ± SE	
				Cellularity ($\times 10^{-7}$)[b]	% Viability[c]
CBA/J	1	Saline		6.6 ± 0.66	93.6 ± 1.9
		$NiCl_2$	9.1	7.8 ± 0.79	94.1 ± 2.2
		$NiCl_2$	18.3	5.9 ± 0.85	95.5 ± 0.7
		$NiCl_2$	27.5	3.4 ± 0.22[d]	96.3 ± 1.2
C57BL/6J	2	Saline		6.7 ± 0.50	88.3 ± 1.1
		$NiCl_2$	9.1	5.8 ± 0.34	87.5 ± 1.5
		$NiCl_2$	13.7	4.4 ± 0.45[d]	86.1 ± 1.8
		$NiCl_2$	18.3	5.8 ± 0.74	90.6 ± 1.5
C57BL/6J	3	Saline		6.8 ± 0.96	91.6 ± 1.3
		$NiCl_2$	18.3	4.9 ± 0.52	88.1 ± 1.7
		$NiCl_2$	27.5	5.0 ± 0.48	92.3 ± 1.1
		$NiCl_2$	36.6	4.9 ± 0.40	88.2 ± 0.7

[a] Treatment as described in Table 1.
[b] Mean number of nucleated cells per spleen.
[c] Percent viable nucleated cells (cell excluding trypan blue) per spleen.
[d] $P < 0.05$, Dunnett's t test compared with saline-injected mice.

mice was due to altered lysis kinetics, spleen cells from $NiCl_2$ treated CBA/J mice (18.3 mg/kg) were cultured with target cells over a period of 24 hr. As shown in Fig. 1 the NK response of spleen cells from $NiCl_2$-treated mice was significantly lower at each time point compared with that of saline-injected controls. These

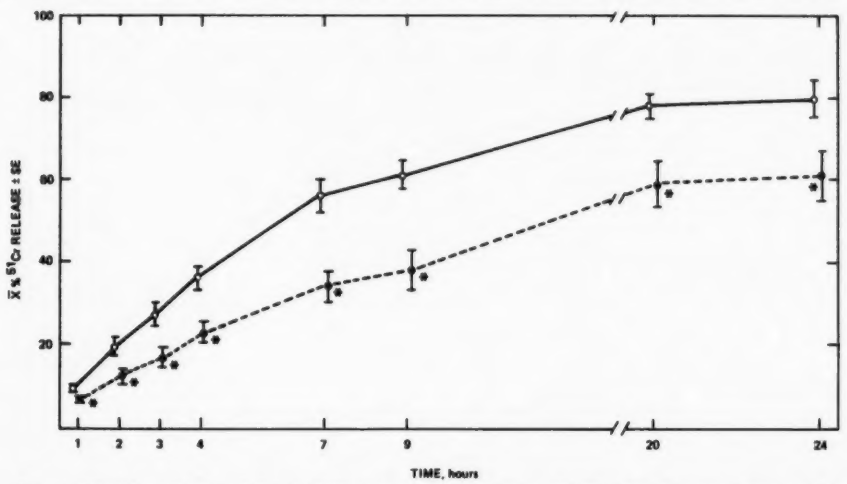

FIG. 1. Kinetics of NK activity in $NiCl_2$-treated mice. Groups of 6 CBA/J mice were given a single intramuscular injection with 18.3 mg/kg $NiCl_2$ (●) or saline (○) 24 hr prior to assay. The amount of ^{51}Cr released from triplicate cultures from each mouse was measured at an E:T ratio of 25:1 with YAC-1 targets at the times indicated. An asterisk indicates $P < 0.05$, Student's t test.

results do not entirely rule out a delay in the expression of normal NK activity; however, a more likely explanation is a decrease in the relative proportion of NK cells in $NiCl_2$-treated mice.

Experiments were performed to determine if the $NiCl_2$-induced reduction of NK activity was due to the generation of suppressor cells. Table 3 shows the results of one of the studies in which a 50% reduction in NK activity was observed in $NiCl_2$ (18.3 mg/kg) vs saline-injected mice. The addition of graded numbers of spleen cells from $NiCl_2$-injected mice (putative suppressor cells, S) to spleen cells from saline-injected mice (effector cells, E) slightly reduced the NK activity at the highest S:E ratio (i.e., 2:1). However, when equal numbers of thymocytes (filler cells) were added to saline effector cells a comparable reduction of NK activity was observed at an S:E ratio of 0.5:1 and significant reduction was observed at an S:E ratio of 2:1. These results indicate that $NiCl_2$ treatment does not generate suppressor cells that are responsible for the observed reduction in NK activity.

Several experiments were performed to determine the effect that repeated injections of $NiCl_2$ have on NK activity. The results of one such study are shown in Fig. 2. CBA/J mice were injected 5 days/week for 2 consecutive weeks with 1.8, 3.6, or 7.2 mg/kg $NiCl_2$ per day. NK activity was determined on the third day following the last injection. The data show that a dose of 18.3 mg/kg administered over a 2-week period significantly reduced NK cell activity compared with saline controls. Reduction of NK activity was comparable at the three doses employed. There was, however, no decrease in the spleen cellularity among the four groups (i.e., 60.2 ± 5.0, 67.0 ± 6.0, 63.0 ± 5.0, and 73.0 ± 8.0 million

TABLE 3
LACK OF SUPPRESSOR CELLS FOR NK ACTIVITY IN SPLEEN CELLS FROM $NiCl_2$-TREATED MICE

Source of effector cells[a]	Source of suppressor cells[b]	S:E ratio[c]	$\bar{X}$% ^{51}Cr Release[d] 25:1	50:1	100:1
Saline-treated mice	None		8.4	12.6	17.9
$NiCl_2$-treated mice	None		4.8	6.4	8.9
Saline-treated mice	$NiCl_2$-treated mice	0.25:1	7.3	11.4	17.5
		0.5:1	7.2	11.8	16.8
		1:1	6.1	10.5	15.5
		2:1	6.1	9.8	14.0
Saline-treated mice	Thymus cells	0.25:1	7.3	11.9	15.7
		0.5:1	6.6	10.1	13.6
		1:1	4.6	7.5	10.3
		2:1	3.3	5.2	7.2

[a] Effector cells were spleen cells pooled from groups of 5 CBA/J mice injected intramuscularly with saline or 18.3 mg/kg $NiCl_2$ 24 hr before assay.

[b] Putative suppressor cells were spleen cells pooled from $NiCl_2$-injected mice (18.3 mg/kg) or thymus cells from saline-injected mice.

[c] Suppressor-to-effector cell ratio.

[d] Mean of quadruplicate cultures at effector-to-target cell ratios of 25:1, 50:1, and 100:1, YAC-1 targets.

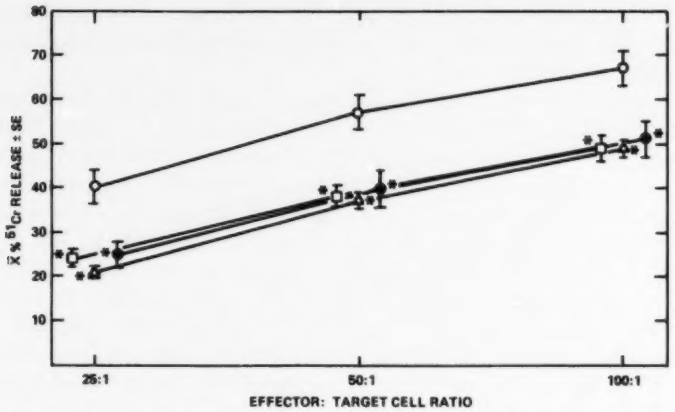

FIG. 2. The effect of repeated $NiCl_2$-injections on NK activity. Groups of 8 CBA/J mice were injected 5 days/wk for 2 consecutive weeks with saline (○), 1.8 mg/kg $NiCl_2$ (□), 3.6 mg/kg $NiCl_2$ (△), or 7.2 mg/kg $NiCl_2$ (●). Three days following the last injection mice were killed and the NK assay was performed. An asterisk indicates $P < 0.01$, Dunnett's t test.

cells/spleen for saline, 1.8, 3.6, and 7.2 mg/kg $NiCl_2$ respectively). There was also no difference in the spleen cell viability among the four groups (i.e., approximately 90% viability).

The effect of $NiCl_2$ on *in vivo* NK activity was determined using an organ clearance assay. A single injection of 18.3 mg/kg $NiCl_2$ significantly reduced the clearance of YAC-1 tumor cells from the lungs (Table 4). Mice injected with hydrocortisone, a positive control, also had significantly reduced clearance of labeled tumor cells from the lung. Paradoxically, the spleens of $NiCl_2$-treated mice

TABLE 4
THE EFFECT OF $NiCl_2$ ON THE *IN VIVO* CLEARANCE OF ^{51}Cr-YAC-1 CELLS FROM SPLEEN AND LUNG

Expt.	Treatment	N	$\bar{X}$% *In vivo* recovery of radioactivity at 4 hr[a]	
			Spleen	Lungs
1	Saline	15	0.68 (0.54–0.97)[c]	0.44 (0.30–0.65)
	$NiCl_2$[b]	15	0.44 (0.23–0.76)[d]	1.15 (0.53–2.95)[d]
2	Saline	12	0.58 (0.35–0.88)	0.50 (0.35–0.83)
	$NiCl_2$[b]	12	0.39 (0.24–0.56)[d]	0.78 (0.45–1.29)[d]
3	Saline	11	0.56 (0.26–1.02)	0.89 (0.30–1.45)
	Hydrocortisone[e]	12	0.49 (0.27–0.68)	2.57 (1.23–3.33)[d]

[a] Geometric mean percent recovery.
[b] CBA/J mice injected intramuscularly with 18.3 mg/kg $NiCl_2$ 24 hr before assay.
[c] Range of values.
[d] $P < 0.01$, Student's t test.
[e] Two consecutive daily intraperitoneal injections of 10 mg hydrocortisone in CBA/J mice. Mice assayed 24 hr after second injection.

and hydrocortisone-treated mice demonstrated enhanced tumor cell clearance with $NiCl_2$-treated mice in both Experiments 1 and 2 having significant ($P < 0.01$, Student's t test) enhancement of clearance.

The development of lung tumor colonies in mice injected with B16-F10 melanoma cells following $NiCl_2$ treatment is shown in Table 5. A single injection of 18.3 mg/kg $NiCl_2$ 24 hr before injection of B16-F10 cells significantly increased the number of resulting lung tumors compared with saline controls. Mice treated with cyclophosphamide, which served as positive controls, also had significantly greater numbers of lung tumors compared with saline-injected mice. These results indicate that the reduction of NK cell activity in mice injected with $NiCl_2$ can lead to increased susceptibility to a transplanted tumor.

DISCUSSION

The results presented here confirm and extend our earlier work on the effects of $NiCl_2$ on NK cell (Smialowicz *et al.*, 1984). A single intramuscular injection of 18.3 mg/kg $NiCl_2$ caused a significant reduction in NK activity of splenocytes from CBA/J (high NK responsive) and C57BL/6J (intermediate NK responsive) mice. This same dose of $NiCl_2$ also caused a significant reduction in the clearance of tumor cells from the lungs of $NiCl_2$-treated mice and significantly enhanced the development of B16-F10 lung tumor colonies. Exposure to 18.3 mg/kg $NiCl_2$ administered over a 2-week period caused a significant reduction (approximately 50%) in NK activity comparable to that observed following a single injection. Unlike the single injection regimen in which a dose–response effect of $NiCl_2$ on NK activity was observed (Table 1), comparable suppression by cumulative doses of 18.3, 36.6, and 72 mg/kg $NiCl_2$ was observed (Fig. 2).

The reduction of NK activity at a dose of 18.3 mg/kg does not appear to be due to an overall reduction in spleen cellularity. While there was a reduction in spleen cellularity at a dose of 18.3 mg/kg this was not significant ($P > 0.05$).

TABLE 5
ENHANCEMENT OF THE DEVELOPMENT OF LUNG TUMOR COLONIES FOLLOWING INJECTION OF B16-F10 MELANOMA CELLS IN $NiCl_2$-TREATED MICE

Expt.	Treatment[a]	Dose (mg/kg)	$\bar{X}$ Tumors/lung ± SE
1	Saline		4.8 ± 0.6
	$NiCl_2$	18.3	>100[b]
2	Saline		6.7 ± 1.3
	$NiCl_2$	9.1	13.6 ± 2.2
	$NiCl_2$	13.7	14.3 ± 4.2
	$NiCl_2$	18.3	63.8 ± 7.6[c]
3	Saline		12.2 ± 2.7
	Cyclophosphamide[d]		53.8 ± 5.8[c]

[a] Groups of 8–10 C57BL/6J mice injected intramuscularly with saline or $NiCl_2$ 24 hr before an intravenous injection of 1×10^5 B16-F10 melanoma cells.

[b] Tumors too numerous to count.

[c] $P < 0.01$, Mann–Whitney U test.

[d] Mice injected with cyclophosphamide (200 mg/kg) 4 days before B16-F10 injection.

However, a higher $NiCl_2$ dose (i.e., 27.5 mg/kg single dose) in CBA/J mice caused a significant ($P < 0.05$) reduction in spleen cellularity. This is in agreement with our earlier results in which the spleen-to-body weight ratio of CBA/J mice injected with 27.5 mg/kg $NiCl_2$ was significantly reduced compared with saline controls (Smialowicz *et al.*, 1984). The lack of a significant decrease in spleen cellularity at other doses or for another strain of mice does not mean that there may not be a reduction in the relative percentage of NK cells in the spleens of $NiCl_2$ treated mice. $NiCl_2$ may be selectively cytotoxic for certain cell populations (e.g., NK cells and T lymphocytes). Furthermore, since NK cells are thought to represent less than 5% of the total splenic population (Roder and Kiessling, 1978) a reduction in the relative number of these cells would not necessarily be reflected by significantly reduced spleen cell counts.

NK activity is not completely eliminated by a single injection of 18.3 mg/kg $NiCl_2$. A residual amount of NK activity remains which can effectively lyse target cells when cultured longer than the normal 4-hr period (Fig. 1). Further work is necessary to determine if there are inherent defects in NK cells from $NiCl_2$-treated mice, as has been observed in certain strains of mice and in certain human diseases (Roder and Durve, 1979; Haliotis *et al.*, 1980), or if this reduction is due solely to decreased numbers of NK cells following nickel treatment.

$NiCl_2$-induced NK suppression does not appear to be mediated by suppressor cells. The addition of spleen cells from $NiCl_2$-treated mice to normal spleen cells did not inhibit NK activity any more than the addition of filler cells (thymocytes). In fact, coculture with filler cells from the thymus, which has recently been shown to contain supressor cells (Zöller and Wigzell, 1982), caused an inhibition of NK activity, the magnitude of which exceeded that of cells from $NiCl_2$-treated mice (Table 3). Using this criterion $NiCl_2$ does not induce suppressor cells as defined by Hochman and Cudkowicz (1979). In addition these results provide additional evidence that $NiCl_2$-induced NK suppression does not appear to be mediated by the release of corticosterone (Smialowicz *et al.*, 1984). Hochman and Cudkowicz (1979), demonstrated that the spleens of hydrocortisone-treated mice contained NK suppressor cells which, when removed by adherence to or phagocytosis of carbonyl iron particles, partially restored NK activity. Furthermore, Hochman and Cudkowicz (1979) also reported that NK activity from untreated mice was suppressed *in vitro* by splenocytes from hydrocortisone-treated mice. We did not observe this suppression with cells from $NiCl_2$-treated mice in the present study.

The clearance of radiolabeled tumor cells from the lungs of $NiCl_2$-treated mice was significantly reduced compared with saline controls. The fact that clearance from the spleen of $NiCl_2$-treated mice was enhanced is difficult to explain. However, clearance of YAC-1 cells from the spleen of hydrocortisone-treated mice was also enhanced. It should be noted that it is generally accepted that clearance of tumor cells from the lungs is a more predictive indicator of *in vivo* NK activity (Riccardi *et al.*, 1980a and b). The clearance of tumor cells *in vivo* has been shown to correlate well with the levels of *in vitro* NK activity (Riccardi *et al.*, 1980a and b). In the present study we have demonstrated a parallel between the effect of $NiCl_2$ treatment on *in vitro* NK activity and *in vivo* clearance of tumor cells from the lungs.

As a further demonstration of the *in vivo* effect of $NiCl_2$ on NK activity we

have shown that this metal salt enhances the development of lung tumor colonies in mice injected with the B16-F10 melanoma. The B16-F10 melanoma was selected for its high lung colonization potential (Fidler, 1978). It has been demonstrated that the effector cells active *in vitro* and *in vivo* against this tumor are NK cells (Hanna and Burton, 1981; Hanna, 1982; Hanna and Schneider, 1983). A single injection of 18.3 mg/kg $NiCl_2$ given 24 hr prior to B16-F10 tumor cell injection significantly enhanced the number of tumor colonies in the lungs. Cyclophosphamide, a known suppressor of NK activity (Hanna and Burton, 1981), caused a similar enhancement of B16-F10 lung tumors.

In earlier work (Rogers *et al.*, 1983), we showed that suppression of NK activity by $NiCl_2$ can be reversed by the injection of a single dose of $MnCl_2$. The injection of $MnCl_2$ either simultaneously or 1 day before $NiCl_2$-injection caused an enhancement of NK activity. Injection of $MnCl_2$ 1 day after $NiCl_2$, however, caused a reduction in NK activity comparable to that caused by $NiCl_2$ alone. It appears that suppression of NK activity by $NiCl_2$ may be reversed by $MnCl_2$. It is interesting to point out that Sunderman *et al.* (1976) and Sunderman (1979) reported that concurrent manganese treatment reduced the tumor incidence in rats injected with nickel subsulfide.

There is increasing evidence from epidemiological studies that humans exposed to dust in nickel refineries have an increased risk of cancers of the nasal cavities and lungs (Sunderman, 1981). The potential enhancement of tumor development in animal models following nickel treatment has also been reported. For example, an increased incidence of spontaneous lung adenomas in strain A mice given repeated parenteral injections of nickelous acetate has been reported by Stoner *et al.* (1976). Increases in the incidence of sarcomas were reported to have developed at the site of a single intramuscular injection of nickel subsulfide in mice (Sunderman, 1979) and in rats (Sunderman, 1976). Furthermore, rats given a single intrarenal injection with nickel subsulfide had a higher incidence of renal cancers (Sunderman *et al.*, 1979). While one might postulate a potential link between the immunosuppressive effects of nickel, in particular the effects on NK activity, and its potential for enhancing tumor development, further research is needed before such a generalization can be made.

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